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Using a bio-inspired model to understand the evolution of the remora adhesive disk

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ABSTRACT

Using a Bio-Inspired Model to Understand the Evolution of the Remora Adhesive Disk

by
Kaelyn Gamel

Manmade adhesives often fail on wet, compliant surfaces, which can result in poor performance when attaching sensors in medical, defense, and research situations. However, a number of fishes have evolved adhesive discs that allow adhesion to surfaces under challenging wetted conditions. A remarkable evolutionary advancement is found in the family of *echenidae*, colloquially known as the remora. In particular, the remora fishes have the ability to attach to wet, compliant bodies under high shear conditions for extended periods of time. This research addresses the lack of underwater adhesives by using remora adhesion as a bioinspired model. Evolution has taken part on this family of species, allowing them to have a biologically advanced suction cup (adhesive disk), which is dorsally located. This adhesive disk includes a complex and integrated bone and muscle structure that enhances the adhesion of the remora on rough surfaces.

Using 3D design, an artificial adhesive disk was constructed to help understand the integration of the adhesion process on different surfaces. The use of AutoCAD's 123D design was used to structurally replicate the remora adhesive disk. Forms lab and Makerbot were the 3D printers used to create this remora disk in real 3D space. Rubber replicated surfaces were created to test the remora 3D disk. These molds are made up Tin-cure silicone rubber, which

were produced to replicate different surfaces such as sharkskin, sand paper, dense arrangement of stone and a smooth surface. Using these different surfaces to test the adhesive disk, our final goal of the work was to produce an adhesive platform suitable for the attachment of instruments for biologging and telemetry research under challenging conditions. Using computed microtomography (microCT) scans of hard and soft tissues of the remora disc as a starting point, we developed a 3D-printed model for experimental testing. Ongoing testing has confirmed the model adhesive disk is the lowest functional module of this hierarchical system that will produce adhesion to both smooth-compliant and rough surfaces.

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Kaelyn Mykel Gamel

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APPROVAL PAGE

Using a Bio-Inspired Model to Understand the Evolution of the Remora Adhesive Disk

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Mom and Dad,

Thank you for all of the love and support you gave me throughout my life. You guys are truly an inspiration and I wouldn't have made it through college without you guys.

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CHAPTER 1

INTRODUCTION

1.1 Objective

The objective of this thesis is to describe the unique adhesive disc of the remora and understand its mechanical properties. Additionally, this work describes the design, fabrication, and testing of a mechanical model of the remora adhesive disk, which has the potential ability to be used in many different applications, such as adhesives for sensors in medical, defense, and research situations.

1.2 Background Information

The family of Echeneidae includes three genera (Remora, Echeneis, Phtheirichthys) and contains of total eight different species. Remoras are a group of fishes that have an elongated body, small, cycloidal scales, and a flat head. The trademark of the family is the dorsally located elliptical-shaped adhesive disk. Echeneidae, derived from Greek etymology, means to hold (echein) ships (nays), but is also believed to be a misinterpretation of “holding on to ships” (Copenhaver, 1991). The remora is the only known fish to have derived an adhesive disk from a dorsal fin, which normally helps stabilize a fish while swimming (Drucker and Lauder, 2015). The origin of the remora’s adhesive disk has been debated (O’Toole, 2002; Richards, 2005; Fulcher and Motta, 2006), but recent studies provide evidence to prove the development of the adhesive disk from the dorsal fin (Britz and Johnson, 2012). This occurs during the early development of a remora. The use of cell mapping to understand where each cell derives from in growth development can be used to compare the remora's development to other closely related species. Additionally, it is found that the remora's early stages of dorsal development resemble those of the spinous dorsal fin and its supporting skeleton in Morone (Britz et al. 2012, Fulcher and Motta 2006). Also, this suction disk is composed of modified proximal, fused medial and distal pterygiophores, which generate the consecutive rows of lamellae (Fulcher and Motta, 2006).

A few other fishes have suction pads, but the suction pad is located ventrally, derived from the pelvic fins, and do not generate friction for attachment. This includes the snailfish (Liparis montagui; Gibson, 1969), clingfish (Wainwright, et al. 2013), lumpsuckers (Davenport and Thorsteinsson, 1990) and river loaches (De Meyer and Geerinckx 2014). The remora's suction pad is unique as compared to these because remoras have the ability to attach to many different hosts (surfaces) of different roughness and compliance, and have 10 to 28 transverse moveable lamellae within the disk area (Nelson, J.S 1984) that generate friction and help with drag and shear forces to keep the remora from experiencing slippage. These surfaces vary depending on the species or object to which the remora is attaching. Some examples of the host species are sharks, rays, other pelagic fish, sea turtles, dolphins, divers, buoys, ship hulls, and concrete (Beckert, 2015). The remoras attach themselves to these larger hosts for a variety of reasons, such as protection from predators, efficient transportation, improving reproduction, and increasing gill ventilation. Attaching to live hosts also allows the remora to feed off remainders of the host’s prey (Beckert, 2015; Fertl and Landry, 1999).

The adhesive disk has a complex hierarchical structure that allows them the ability to attach and detach from several types of wet surfaces. Within this structure there is a highly organized system of bones and muscles. The outer fleshy lip creates a suction-cup like seal (Britz and Johnson, 2012; Beckert et al. 2016a,b). This fleshy lip is composed of soft tissue, allowing the lip to suction from rough to smooth surfaces and maintain a seal. Located in the fleshy lip is a series of lateral line innervated sense organs, which are nerve endings that are sensitive to touch. These nerve endings can be compared to mammals Meissner’s corpuscles, which play an important role in the attachment period of the remoras (Kuhn, 1959). Understanding the complex anatomy and how the suction disk has evolved over time will allow for the simplification and application of the fundamental function of the adhesion process. Conclusively, understanding the anatomical structures and their roles within the disk itself permits for simplistic replication of the remora disk.

1.3 Echeneidae Evolution

Remora evolution is important to understand when investigating the function of the adhesive disk and its importance in the Echeneidae family. Freidman et al. (2013) explains in the paper “An early Fossil remora (Echeneoidea; Echeneidae) reveals the evolutionary assembly of the adhesion disc” were able to use past research that show the developmental patterns, ontogentic work, and segmented construction of the adhesion disks. These works show that this dorsally located adhesive structure was derived from other acanthomorphs (Figure 1.1). Acanthomorpha is a diverse taxon of teleost fishes (spiny-ray fishes).

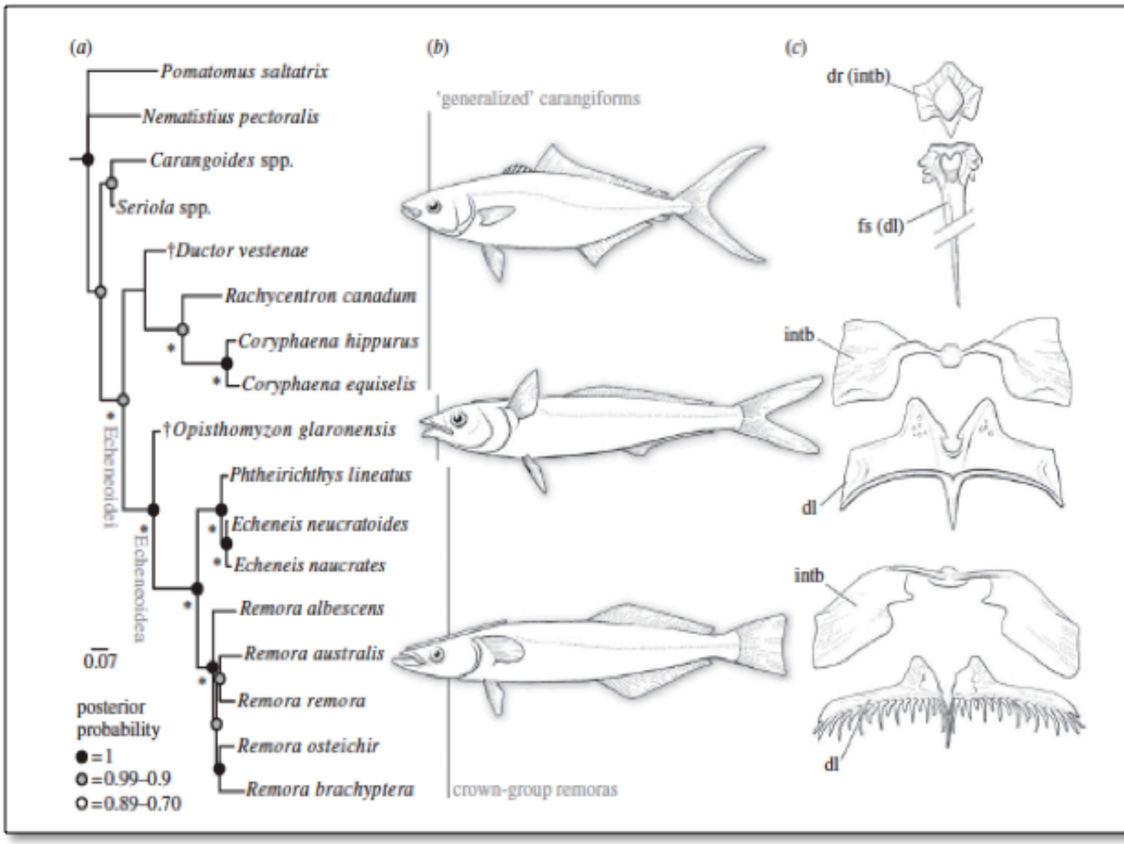

Figure 1.1 Is the current phylogenetic tree (A) of the clade Echeneidae and its close ancestral relatives. (B) Shows a general body morphology of the Echeneidae and the close relatives. (C) Correlates with B and illustrations the disc lamella of the remora and its close relatives. Source: Friedman et al., 2013.

This clade has hollow and unsegmented spines at the anterior portion of the dorsal and anal fins, which allows for numerous evolutional divergences within the clade. Furthermore, to finalize the phylogenic tree analysis, the use of the Bayesian inference analysis was used. This analysis is a 'total- evidence' dataset that uses data from the remoras and its closest biological relatives. The data uses information from the species morphology, development, fossils, and genetic sequencing.

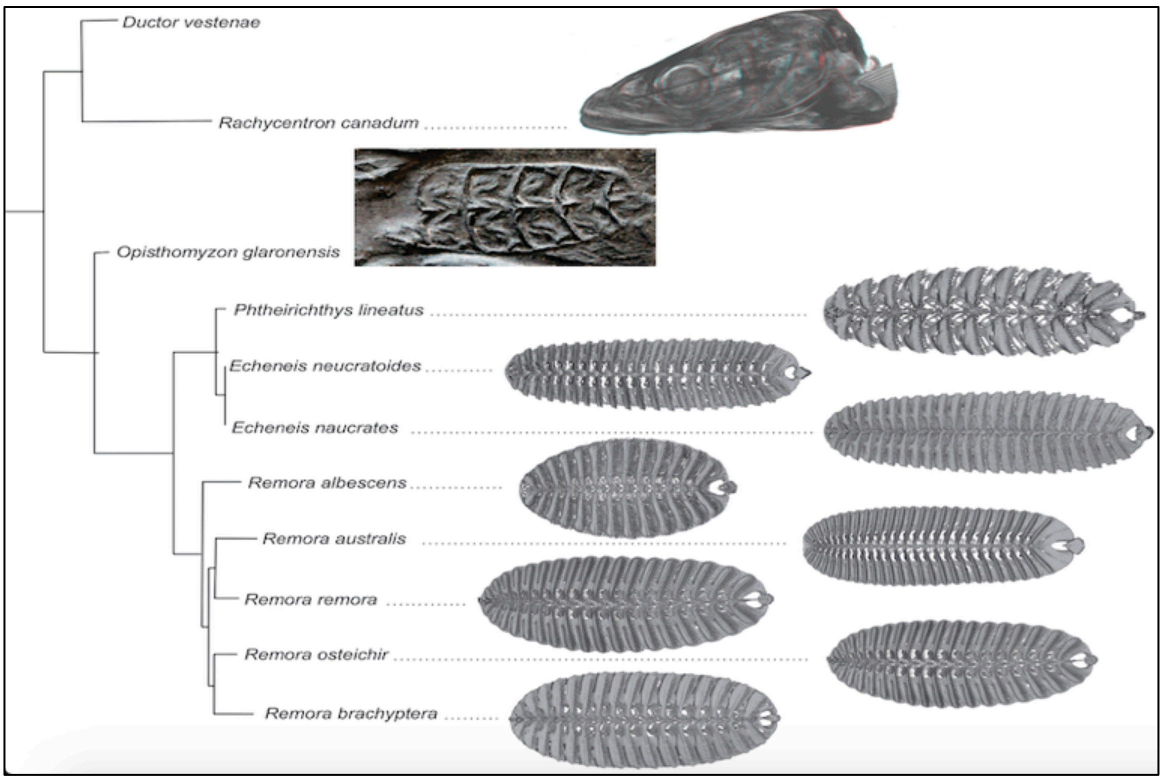

Figure 1.2 Micro-CT scans of the current phylogenetic tree, used from Friedman et al. (2013) phylogenetic tree. Source: B.E. Flammang, unpublished data.

Using Friedman et al. (2013) phylogenic tree, Flammang (unpublished data, personal communication) constructed the same phylogenic tree with micro-

CT scans of the various species' adhesive disks (Figure 1.2). Having these CT scans of the remora adhesive disk, allows one to compare closely related species. This phylogenic tree confirms that there are many successful models of disk adhesion while maintaining different disk morphologies.

1.4 Remora Disk Morphology

On the top of the remora's head is an elliptical adhesive disk, which contains many integrated structures. The structures include spinules, louvered-paired pectinated lamellae, wing-like intercalary bones, interneural rays and an outer fleshy lip (Britz and Johnson, 2012). The outer fleshy lip, located in Figure 1.3, will generate a seal that will act as the suction cup of the adhesive disk.

Figure 1.3 Picture of adhesive disk and the arrow is pointing to the outer fleshy lip.

It is important to recognize the middorsal septum, which separates the two sides of the disk. This septum, made of connective tissue, runs ventrally of the disk and continues down to the neurocranium (Fulcherand Motta, 2006). Contributing to the septum is where the middle spinule connects the opposing lamellae and creates a bifurcated base (Figure 1.4).

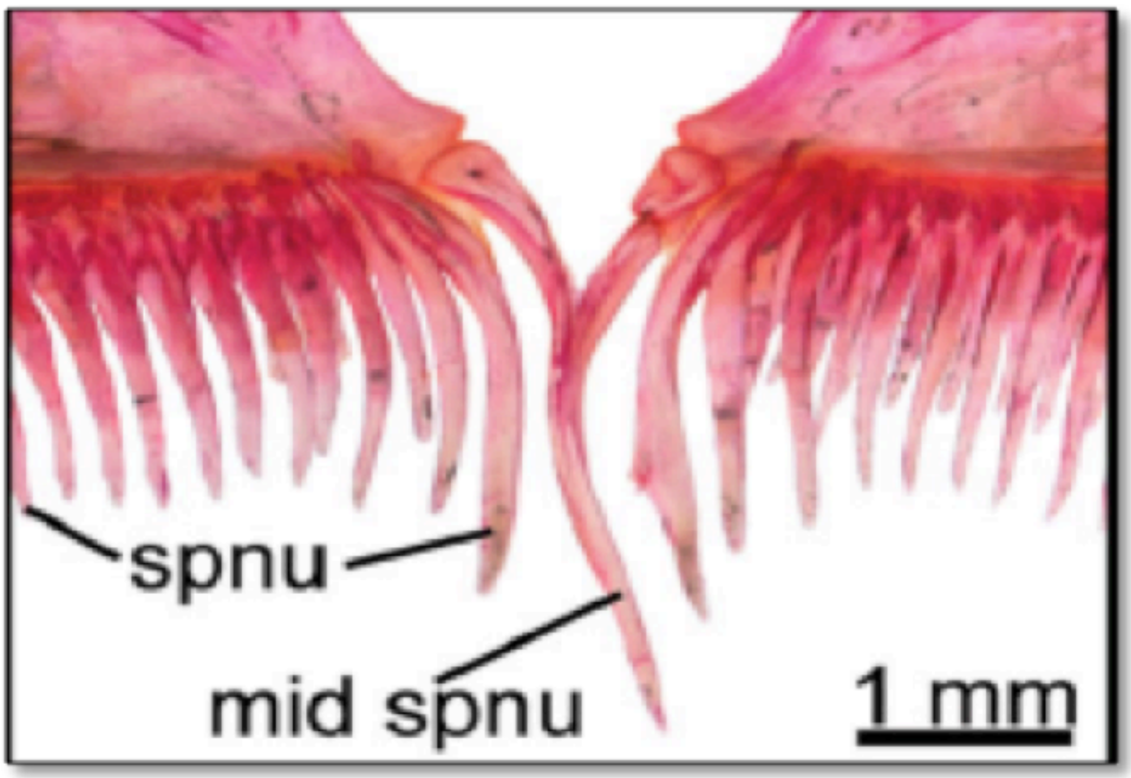

Figure 1.4 Close up of the mid spnu (Middle spinule) and pectinated lamellae. Source: Britz and Johnson, 2012.

Figure 1.5B shows the spinules that will generate resistance and are fit to slide into the host’s scales and/or divots. The spinules help keep the remora from slipping off due to drag and other forces. As shown by Beckert et al. (2015), the increase in the number of engaged spinules in contact with the rough surface increases friction significantly. These spinules are long cylinders with a cone-

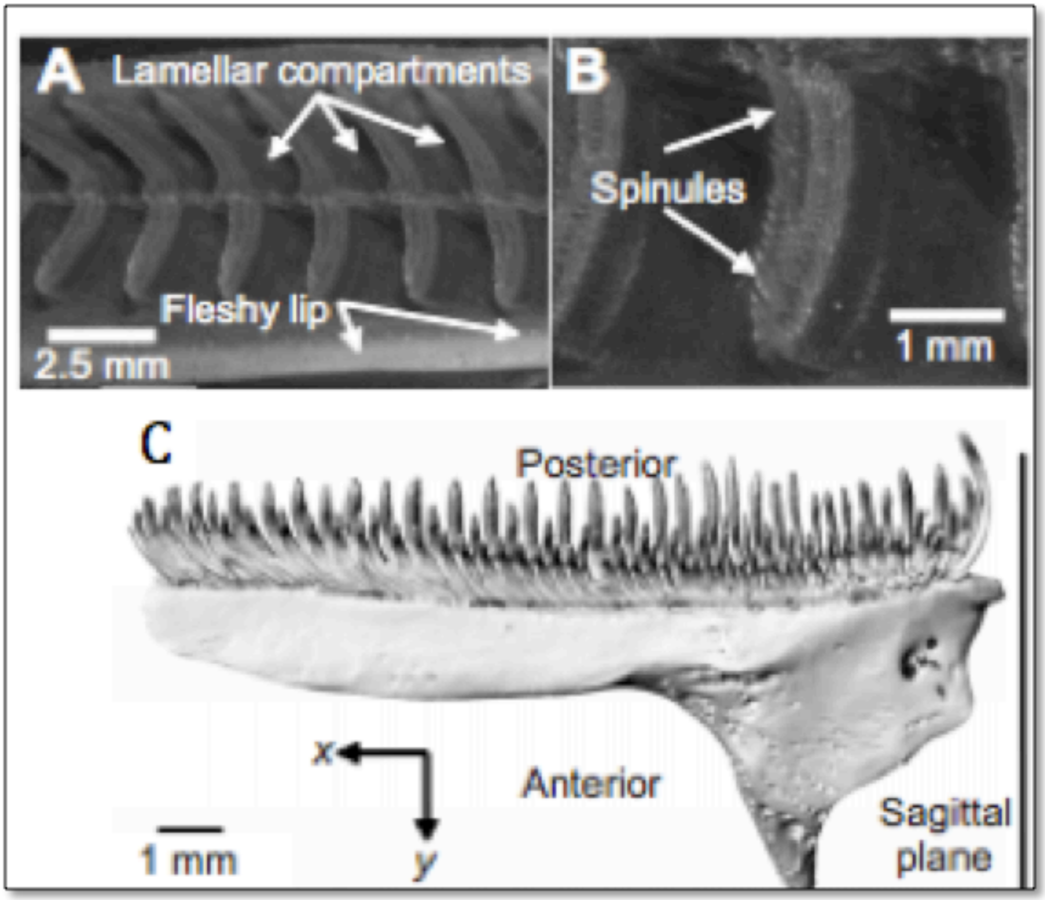

Figure 1.5 shows the lamellar compartments and the outer fleshy lip (A). (B) is a zoomed in version of A, but this helps demonstrate that the spinules are small in size, compared to the lamellae (B+C). (C) is a micro CT scan of on singular lamella. Source: Beckert, Flammang, and Nadler paper published in 2015.

shaped structure at the tip. This cone-shaped structure at the end of the spinules helps engage and generate friction to reduce slippage off of the host. Figure 1.5C is a micro-CT scan of a lamella and spinules, which shows the anatomical structure and the interaction between the lamella and the spinules.

CT-scans use x-rays to make detailed pictures of tissues, bones and cartilage, which are combined to generate 3D image. Furthermore, B. Flammang used micro-CT scanning of a remora's morphological disk and dissected the

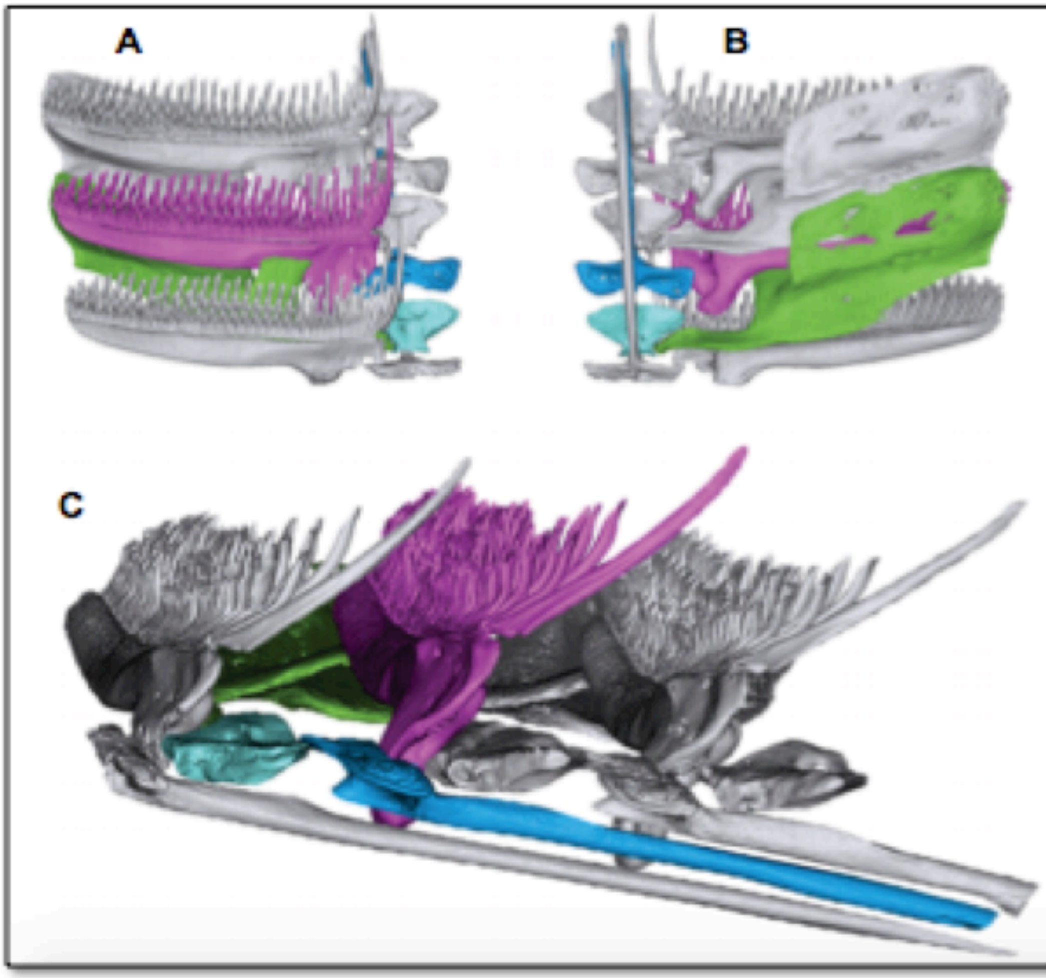

Figure 1.6 Bones of the remora cranial disc. pectinated lamella with spinules (magenta), intercalary bone (green), interneural ray (blue), and anterior intercalary bone (teal). Part A shows the dorsal view of the anatomical structure within the remora adhesive disk. B is the ventral view of the adhesive disk. C shows the lateral view of the adhesive disk. Images from B. Flammang (unpublished data).

image using an image processing software, Mimics (Materialise USA). Mimics take the micro-CT scans and joins it in a 3D model, and allows for a detailed view of each bone structure. This is also very useful when looking at the intricate way the bones intertwine and work together to perform a certain task (Figure 1.6) and comparing the anatomy of different species 1.2).

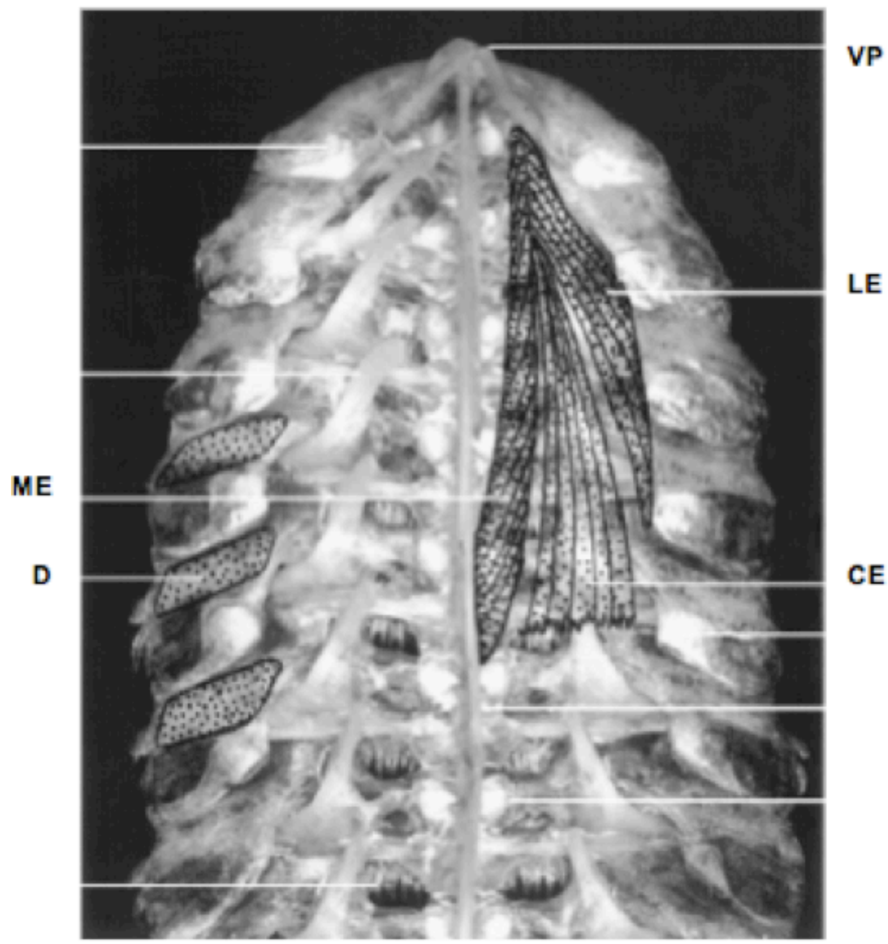

Figure 1.7 Simplified version, showing the erector and depressor muscles within the adhesive disk of the remora E. naucrates. (VP) ventral process; (LE) lateral erector; (CE) central erector; (ME) Medial erector; (D) depressor muscle. Source: Fulcher and Motta 2006.

Within Figure 1.6, the individual bones of the disc were segmented and a variety of colors was used to represent the different individual bones. The magenta colored bone is the pectinated lamella where the green shaded bone is the intercalary bone. The teal is the anterior intercalary bone, which is in front of the interneural ray, which is colored blue. The pectinated lamella (magenta) is hinged tightly between the intercalary bone (green), which allow for accurate control over the angle in which the pectinated lamellae take when adhering to surfaces. Attached ventrally to the intricate bone structures, muscle tissue is present throughout the adhesive disk, allowing for precise control during the attachment and detachment process (Figure 1.7).

There are three main erector muscles throughout the disk lateral, central and medial erectors and a depressor muscle. These muscles are also derived from the muscles that are used to control the dorsal fin, or the pterygiophores (Fulcher and Motta, 2006) The erector muscles take part in engaging the lamellae and spinules to lock in on to the surface, which the remora is adhering. These erector muscles work in conjunction with a tendon that connects to the ventral process (VP) as shown in Figure 1.7. The lateral erector (LE) is connected to the distal part of the intercalary bone. The central erector (CE) is a fan shaped muscle, which comes ventral of the disk and attaching to the tendon. The medial erector (ME) attaches from the anterior intercalary bone and concludes with the other erectors at the tendon. Contributing to the muscles located in the adhesive disk is the lateral depressor (D). This depressor muscle pulls the underside of the distal intercalary bone, which allows the lamellae to relax, lay flat or disengage the adhesive disk. When the lamellae are erect this allows for an increase in sub-ambient pressure within the disk itself. Comprehending the functionality and the anatomical and physiological structure of the remora adhesive disk will give insight into redesigning and advancing the replica disk.

1.5 Remora Attachment Process

Remoras face many problems when attaching to any type of surface while being submerged in water. Additional to the success of creating a seal on rough surface, the remora has to produce a sub-ambient pressure that creates the suction element of the suction seal. To help reduce potential slippage the remora has a preference in where they will adhere to on the hosts' body. It has been suggested that remoras do this to minimize interference with the hosts' habitual behaviors and/or for hydrodynamic purposes. Remoras attempt to discreetly attach to the host. The remoras tend to avoid sensitive areas such as eyes and mouth. This is to minimize any host discomfort and maintain the symbiotic relationship (Silva and Sazima, 2008). The remoras seem to have a preference in location of attachment, which leads researchers to test if the remoras are taking advantage of the host's fluid boundary layer to generate less drag. Unfortunately, there was little correlation between the attachment area and the reduction of drag (Beckert et al. 2016A), which leads researchers to believe there is an alternative benefit to the preference of attachment sites. An alternative may be remoras desire to maintain suction seal for long periods of time, and therefore favor spots on the host where the tissue is more rigid.

Additionally, the remora has evolved an efficient body shape to decrease drag forces when adhering to a host. As seen in Beckert et al., (2016A), tests were done in a flume tank to understand the relationship between drag and body shape. Within the flume tank, the remora was attached to a surface and the flume produced a water current to model fluid flow during attachment periods and host motion. Using laser and camera technology, water and small particles flowed around the remora showing a fluid boundary layer between the water and remora. The results were displayed in a video showing the drag forces the remora was subjected to while being attached to a host. With these results, Beckert et al. (2016A) concluded that an increase in body length caused a decrease in ratio of drag to frictional forces.

In addition to a well-developed shape, the remora also produces mucus on top of the adhesive disk. Many fish produce mucus to reduce frictional forces when swimming (Shepard, 1994). The remora produces mucus to create an airtight seal with the host. Mucus helps reduce the fluid flow around the remora’s body, thus further reducing friction. The remoras mucus, when compared to other teleosts, is significantly more viscous (Beckert et al. 2016B). Having an increase in viscosity decreases the amount of fluid flow through the remora suction seal and increases the adhesive abilities of the disk. Moreover, when the remora erects the lamellae, they increase volume between the suction pad and the hosts surfaces. This decreases the sub-ambient pressures producing the suction seal.

The lamellae engage into the local asperities on the surface of the host, which allows the adhesion disk to be resilient against shear movement. This happens because when the lamellae and spinules are engaged they generate a frictional force, significantly reducing the likelihood of the remora being removed by shear forces from the water drag. Having a comprehensive knowledge of the biomechanics associated with the adhesive disk and adhesive process is necessary to create a fully functioning bio-inspired model.

CHAPTER 2

DESIGNING THE REMORA DISK

2.1 AutoCAD 123D Design

AutoCAD 123D Design was used to design the replica of the remora reversible adhesive. 123D Design allows designs to be made in 3D space, which can then be printed utilizing a 3D printer. 123D Design was used to make intricate structures, such as joints, gears, and connections. Understanding the anatomy of the remora adhesive disk and integrating it to design a similar, less complex structure was needed when designing the replica because we wanted to develop a simple model that would be useful in a wide variety of uses and not require power. Designing a simplistic replica of the remora disk was required because it is difficult to replicate muscle flexion and extension without many motors and complex integration; this feature may be included in future iterations but was beyond the scope of this primary study. The lamella showed in Figure (1.5A) has a “T” shape to the cartilage structure. Designing the lamellae to become one long, rectangular structure (Figure 2.1) instead of two half T shapes was done to simplify the 3D replica. The lamellae are attached to the disc body using a ball-in-socket joint to permit the lamellae to rotate with semi-restricted motion. The lamellae needed to have the ability to move, so I designed a joint that allowed the replica lamellae to move on rotation on the x-axis to generate a pitch movement (Figure 2.1, 2.2).

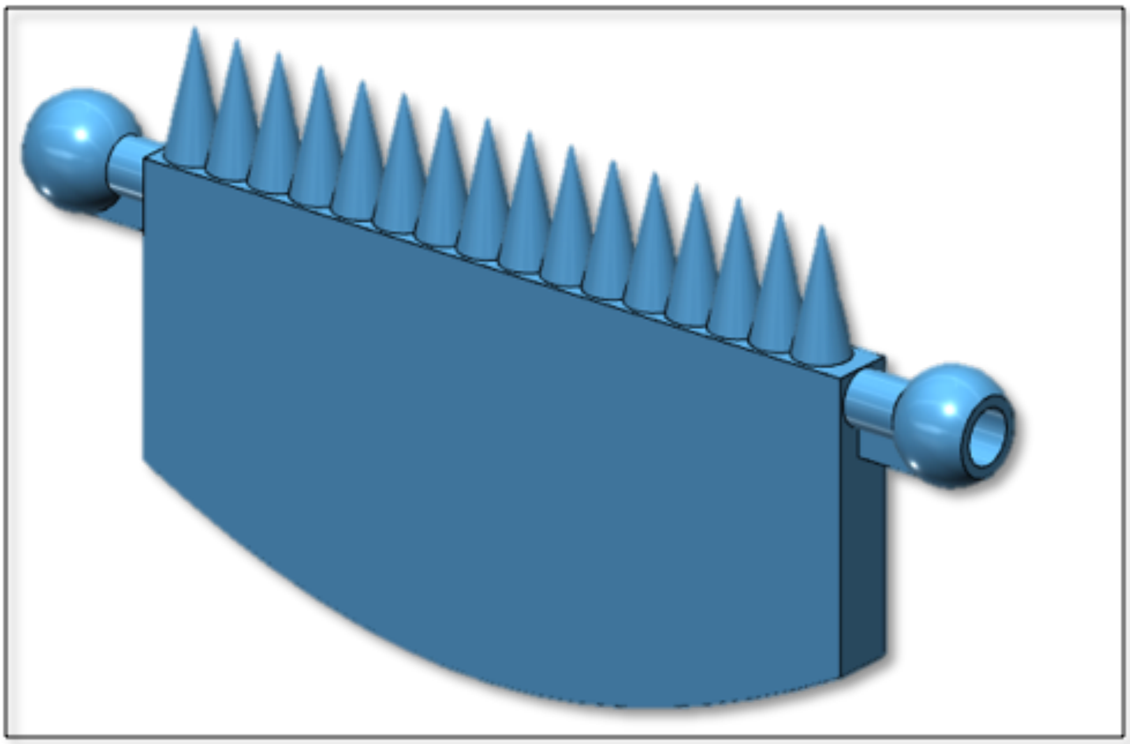

Figure 2.1 simplistic version of the lamellae and spinules used in the remora adhesive disk. Cone like structures were used to redesign the spinules. Ball like structure was used for the Ball and Socket structure. This ball structure is used to attaching the lamellae to the disk.

On the lamellae replica there is a ball like structure on both sides of the lamellae. This is used to slip into the socket joint in Figure 2.2 and allows for the lamellae to stay in place while having a restricted movement. This movement will permit the lamellae to move on a pitch axis and allow for the spinules that are attached to the lamellae; engage into the surface to which it is adhering. The outer fleshy lip (Figure 2.3A) was designed to be more like a suction cup than a fleshy outer lip of the remora simply because this model does not have active muscular control in the outer lip. The body of the adhesive disc (Figure 2.3B) was designed to hold the snap-in lamellae with enough room for them to rotate and engage the surface, with a hook attached to the edge for attachment force measurement testing.

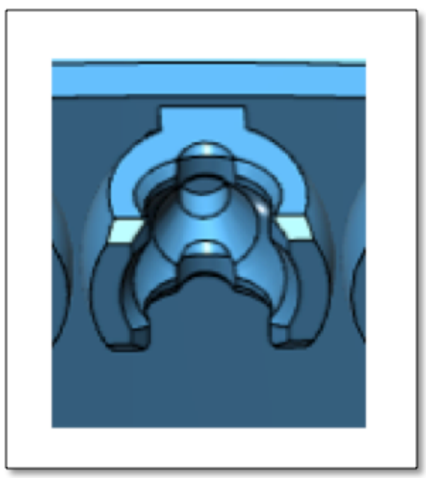

Figure 2.2 Socket part of the joint. This is used to attach the lamellae to the adhesive disk.

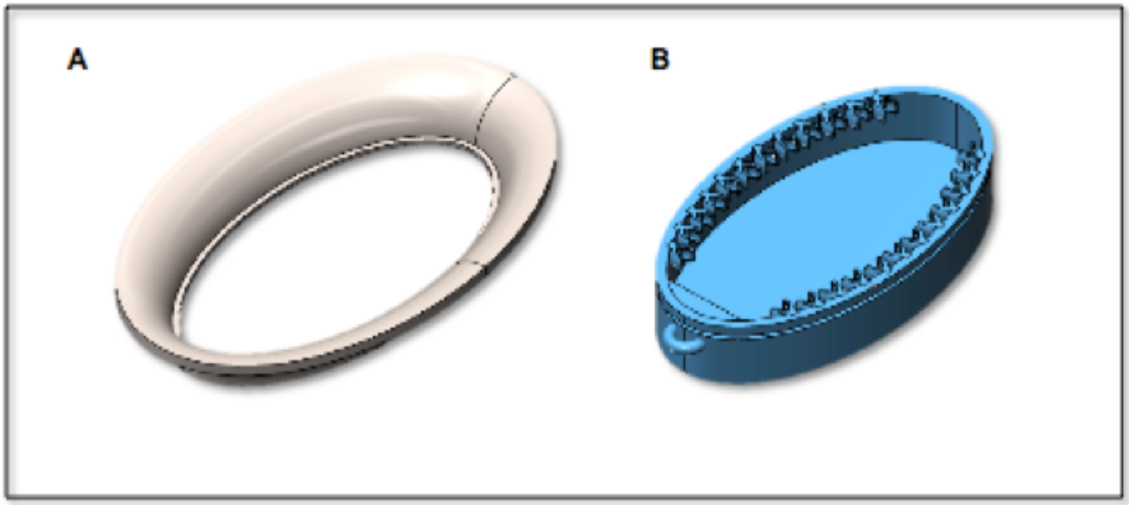

Figure 2.3 Shows the tested replica of the remora adhesive disk. A suction cup-like structure was designed to generate a negative pressure and allow for the adhesive disk to adhere (A). B shows the disk where the lamellae, along with where the string will be attached during testing. There is also a block that does not allow the lamellae to surpass a 90 degree angle.

2.2 Makerbot and Formlab 3D Printers

Two separate 3D printers were used for the model because they used materials with different desirable material properties. The Makerbot Replicator Z was used to print out the beginning works of the remora replica (Figure 6). The Makerbot takes PLA filament and melts it to build a design by incremental layering. After the Makerbot prints the first layer it then continues to move layer-by-layer, printing out a 3D structure. After many different trials, the printer was unable to print small enough joint like structures due to filament size print, which only gets as small as .05 mm. After about 20 trials of redesigning and making sure there were no flaws in the design, moving onto the Formlab printer was required to print more of an articulate design.

The Formlab was used to finalize the 3D design for testing. The Formlab pringer works by having using platform full of sticky, viscous, SLA resin, which is solidified by a laser following the 3D design, file and converts it into a 3D structure. The Formlab printer can print using a variety of resin types and was more favorable for the ability to print a softer, compliant suction disk as well as smaller articulations to accommodate the lamella and spinules.

When printing was complete, the lip and the disk were siliconed to one another allowing for a complete air-tight seal. Lamellae were set in place to create the ball in socket joints on both sides of the disk.

CHAPTER 3

Testing the Remora Adhesive Disk

3.1 Surfaces used for testing

In the biological world, remoras stick to host surfaces with a wide variance in roughness and compliance. To test attachment of our bioinspired disk, silicon molds of different roughness were cast to use as comparable attachment surfaces.. Each mold was shaped in a pad that was 4.86 inches by 3.18 inches, creating a rectangle for the adhesive disc to stick to. The remora model was able to stick to three different scales of roughness. For each surface there was smooth (surface 1), dimpled (surface 2) and rough surface (surface 3). Smooth was less compliant and was smoother than dimpled. Dimpled had faint asperities visible with angled light but these could not be felt by touch. The rough surface was designed by molding 350 grit sand paper on top to attain a roughness similar to that of shark skin. I hypothesized that the disc would not attach as well to rougher surfaces due to seep. There is space between the fleshy lip and the mold due to the roughness divots; this allows air to leak into the disk, creating a loss of sub-ambient pressure, which disengages the suction. I also hypothesized that an increase in lamellae would increase hold time for all surfaces, but particularly surfaces 2 and 3, as the spinules on the lamellae would interact with the local asperities of the molds and generate friction.

3.2 Procedure

Figure 3.1 Pulley system to test the weight and time the suction cup can hold to the surface. The blue pad is the surface where the remora disk attaches.

The mold was placed horizontally, on the surface of a table, while the adhesive disk was placed on top of the pad. Pushing down perpendicularly on the disc, allows air under the disk to escape. Once releasing this creates sub-ambient pressures underneath the disk allowing suction to occur. A pulley system was used to test how much weight or force the disk was able to withstand within one suction trial (Figure 3.1). Each trial consisted of a different amount of lamellae or a different surface. When finding a weight where the disk will eventually fail, tests were done to see the difference of lamellae. Testing 0, 3, 6, 9 lamellae gave the ability to see how the lamellae were contributing to the adhesive process.

3.3 Results and Discussion

Results show as there is an increase in the amount of lamellae there is an increase in adhesion time (Table 3,1). This provides clear evidence that increasing the amount of spinules and lamellae that are engaged to a surface creates a greater frictional force to help the disk resist shear forces. The graph (Figure 3.2) shows that there is potentially a limit to how many lamellae can resist shear without compromising some other aspect of the disc, which would support why remoras don’t have more than 28 lamellae. Further testing will need to be done to provide significant results.

Table 3.1 Results of the Experimental Trials with the Remora disk Model, Varying Surface Types and Number if lamellae

	Number of Lamellae	Number of Spinules	Time Stuck to Surface	Weight Held(lbs)
Smooth	0	0	2.87	10
Smooth	3	73	10.15	10
Smooth	6	126	19.96	10
Smooth	9	186	50.14	10
Dimpled	0	0	30	6.6
Dimpled	3	73	30	6.6
Dimpled	6	126	30	6.6
Dimpled	9	186	30	6.6
Rough	0	0	30	6.6
Rough	3	73	30	6.6
Rough	6	126	30	6.6
Rough	9	186	30	6.6

In terms of evolution, a greater number of lamellae provide an adhesive advantage by enabling the remora to stay engaged to its host for a longer period of time. This advantage allows the remora to travel farther while expending less energy and provides more feeding opportunities. Reproductive success also depends on adhesion to a host; therefore increased adhesion time would lead to an increase in meeting conspecifics and potential reproduction. Having more lamellae is a desirable trait and thus remora with a higher number of lamellae than average will have greater reproductive success and pass this trait to its offspring. For example, if we look at the first known remora, the extinct species Opsithomyzon glaronensis (Figure 1.1,1.2),

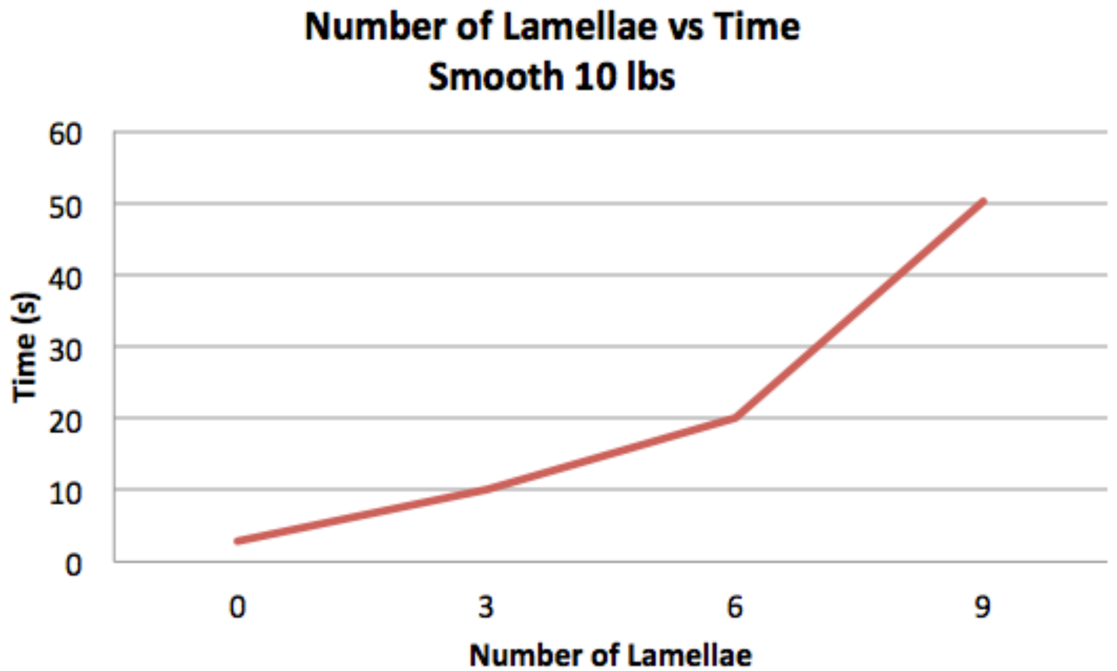

Figure 3.2 Disk adhesion of number of lamellae vs time, holding a 10 lbs weight on surface 1.

we can see a trend suggestive of selection for adhesion performance as the driver for this novel morphology. Opisthomyzon only had six rows of lamellae, which would contribute some adhesion force, but subsequent generations with an increase in lamellae may have benefitted from high fitness as a result.

It was the original intent of this research to have multiple trails to show the relationship between different materials, including the spinules, lamellae, and surfaces. First, trials for all surfaces were done with the disk holding 6.6 lbs. of weight (Table 3.1); this was the amount of weight we anticipated would be sufficient to max out the system. However, the disc did not fail under this amount of shear force, even during the 0 lamellae trial, which leads to the conclusion that the suction alone had the ability to hold the weight and the disk was generating much more force than expected. Increasing the weight to 10 lbs. finally allowed for failure to occur, and showed the differences between the number of lamellae between each trial. When failure occurred the disk would rupture becoming unusable to test in subsequent trails. Unfortunately, this exhausted the number of ready-made disks I anticipated would be needed. The time it takes to manufacture a functioning 3D disk exceeded the allotted amount of time to attain significant results. Between the printing and the adhesive curing process it would take up to a day or more to attain a functioning disk. Adhering the lip to the disk itself required an element of human intervention, which therefore creates slight variances and inconsistences in the adhesion of the lip to the disk. Allowing for minimal human interventions and differences in each disk will allow for more consistent results in the future; this could be accomplished by gaining access to a multi-material 3D printer.

Future works would include in making a multi-material disk where the disk would have fixed lamellae. This multi-material disk will incorporate passive soft tissue biomechanical properties that contribute to the long-term hold of the remora under fluctuating pressure conditions.

3.4 Conclusion

Remoras have evolved a unique way of adapting to their environment to become well equipped for survival. This evolutionarily unique disk creates opportunities for bio-inspired devices. Results from this work show that lamellae pose a viable solution to problems faced by typical adhesion devices in the presence of liquid or changes in ambient pressures. These findings also support the idea that the number of lamellae increased during evolution as selection favored stronger adhesion. Future work will continue to look at more surfaces over a range of greater compliances. A multi-material model, with tissue like properties, will need to be taken into consideration when designing future iterations of the adhesive disk.

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